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Methane production and methanogen levels in steers that differ in residual gain[1,2,3]

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ABSTRACT: Methane (CH_4) gas released by cattle is a product of fermentation in the digestive tract. The 2 primary sites of CH_4 production in ruminants are the reticulum-rumen complex and the cecum. Methane release from cattle represents a 2% to 12% loss of the energy intake. Reducing the proportion of feed energy lost as CH_4 has the potential of improving feed efficiency as well as decreasing the contribution of cattle to greenhouse gas production. Feed intake and growth were measured on 132 fall-born steers for 70 d. Seven steers with extreme positive residual gain (RG) and 7 steers with extreme negative RG whose DMI was within 0.32 SD of the mean intake were selected for subsequent measurements. Enteric CH_4 production was measured via indirect calorimetry. Rumen, cecum, and rectal contents were obtained from steers at slaughter for measurement of in vitro CH_4 production and methanogen 16S rRNA levels. Enteric CH_4 production did not differ ($P = 0.11$) between the positive RG (112 ± 13 L/d) and the negative RG (74 ± 13 L/d) steers. In vitro rumen methane production did not differ between positive RG ($64.26 \times 10^{-5} \pm 10.85 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}) and negative RG ($61.49 \times 10^{-5} \pm 10.85 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}; $P = 0.86$). In vitro cecum methane production did not differ between positive RG ($4.24 \times 10^{-5} \pm 1.90 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}) and negative RG ($4.35 \times 10^{-5} \pm 1.90 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}; $P = 0.97$). Methanogen 16S rRNA as a percentage of the total bacteria16S rRNA did not differ between RG groups ($P = 0.18$). The methanogen 16S rRNA as a percentage of rumen fluid total bacteria 16S rRNA (5.3% ± 3.1%) did not differ from the methanogen 16S rRNA as a percentage of cecum content total bacteria 16S rRNA (11.8% ± 3.1%; $P = 0.14$). The methanogen 16S rRNA as a percentage of the rectum content total bacteria 16S rRNA (0.7% ± 3.1%) was not different from the rumen content ($P = 0.29$) but was less than the cecum content ($P = 0.01$). Methanomicrobiales 16S rRNA as a percentage of total methanogen 16S rRNA did not differ across sample sites ($P = 0.81$); however, steers with positive RG (10.5% ± 1.6%) were more numerous than steers with negative RG (5.1% ± 1.6%; $P = 0.02$). Cattle that differ in RG at the same DMI do not differ in characteristics associated with CH_4 production.

Keywords: cattle, methane, methanogens

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INTRODUCTION

Methane gas released by cattle is a product of fermentation in the digestive tract. The 2 primary sites of methane (CH_4) production in ruminants are the reticulum-rumen complex and the cecum (Murray et al., 1976). There are multiple species of methanogens associated with the generation of CH_4 in the gastrointestinal tract (Yu et al., 2008). Many of the studies that determined levels of methanogens in the rumen were conducted using culture techniques. Culture techniques may underestimate the presence of species that are difficult to culture. A molecular approach would assist in identifying the presence of methanogens that cannot be cultured. Methane release from cattle represents a 2%

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to 12% loss of the energy intake (Johnson and Johnson, 1995). Diet and level of intake contribute to CH_4 production (Flatt et al., 1965; Reynolds et al., 1991; Freetly and Nienaber, 1998; Archibeque et al., 2007). Reducing the proportion of feed energy lost as CH_4 has the potential of improving feed efficiency as well as decreasing the contribution of cattle to greenhouse gas production. One approach has been to select cattle that perform better on lower levels of feed. Nkrumah et al. (2006) and Hegarty et al. (2007) found reduced CH_4 production from steers that had a low residual feed intake (**RFI**). This decrease in CH_4 was associated with a decrease in intake and not a decrease in CH_4 production per unit of feed consumed. Alternatively, cattle that gain more BW on an equal amount of feed (residual gain) may have a different microbiome that results in an increased utilization of the feed. Zhou et al. (2010) determined that cattle that differ in feed efficiency also differ in the level of methanogenic species, which may be a possible mechanism for the reduced CH_4 production. Our hypothesis was that cattle with a positive residual gain (**RG**) would have fewer methanogens or different methanogens than cattle with a negative RG.

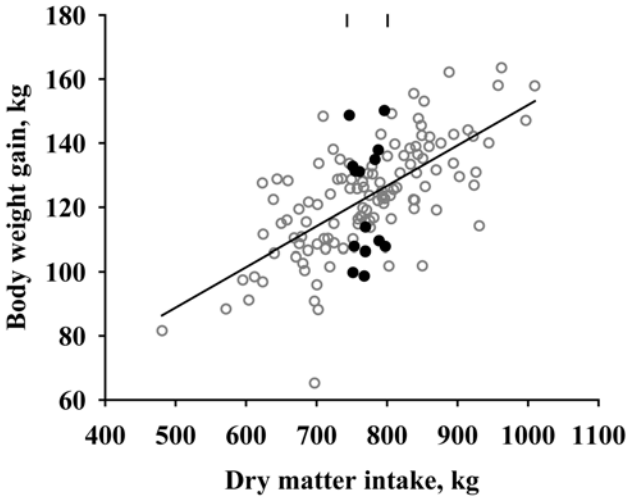

Figure 1. Relationship between BW gain and DMI over a 70-d feeding period: BW gain = *f*(DMI) = (0.1262 ± 0.0128)DMI + (25.7 ± 9.9), R^2 = 0.43. Steers selected for study (solid circles) were within 0.32 SD around the mean. Steers above the regression are positive for residual gain, and steers below the regression are negative for residual gain.

MATERIALS AND METHODS

Cattle

The experiment was conducted to conform with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, 2010) and was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. Feed intake and growth were measured on 132 fall-born steers that were part of a breed evaluation study (Kuehn et al., 2008). Breeds represented included Angus, Beefmaster, Brangus, Brahman, Braunvieh, Charolais, Chiangus, Gelbvieh, Hereford, Limousin, Maine Anjou, Red Angus, Salers, Santa Gertrudis, Shorthorn, and Simmental. Sixty of the steers had the same sire and maternal grandsire breed and included some purebred Angus, Charolais, and Hereford steers. The remaining steers were F_1 progeny from the evaluation breeds. Sires and maternal grandsires were bulls that are being used in industry. At the start of the study, steers were 348 ± 1 d of age and weighed 444 ± 4 kg. Feed intake and growth were evaluated for a 70-d period. Steers had ad libitum access to a diet that as a percentage of DM contained 82.75% dry-rolled corn, 12.75% corn silage, and 4.5% supplement. The supplement contained (% DM) 62.55% limestone, 2.38% NaCl, 32.63% urea, 0.93% trace mineral mix (13% Ca, 12% Zn, 8% Mn, 10% Zn, 1.5% Cu, 0.2% I, and 0.1% Co), 0.56% vitamin mix (A, 8,818,490 IU/kg; D, 881,849 IU/kg; and E, 882 IU/kg), and 0.95% Rumensin-80 (Elanco, Greenfield, IN). Feed intake was measured using an Insentec Roughage Intake Control Feeding System (Insentec B.V., Marknesse, The Netherlands), and total DMI was summed over the feeding period. Steers were weighed on 0, 1, 21, 42, 56, 69, and 70 d of study. Body weight was regressed on days of study for each steer, and total BW gain was calculated from the regression equations. Total BW gain was regressed on total DMI (Fig. 1). Seven steers with extreme positive RG and 7 steers with extreme negative RG whose DMI was within 0.32 SD of the mean intake were selected for subsequent measurements. Steers remained on the same diet following the end of the feed intake and growth study. At 5 to 7 d following the end of the feed intake and growth study, in vivo CH_4 production was measured. Steers were slaughtered 12 to 21 d after the feed intake and growth study. Two steers were harvested per day (1 positive and 1 negative RG steer), and rumen and cecum contents were sampled to determine in vivo CH_4 production and level of methanogens.

In Vivo CH_4 Production

Selected steers were moved to the intensive cattle research facility, housed 1 steer per pen, and fed the same ration used throughout the genetic evaluation period. Approximately 7 d after being moved to the new facility, a single 6-h enteric CH_4 collection was made using indirect calorimeters (headboxes), and gas samples were analyzed for CH_4 using a system previously described (Freetly and Brown-Brandl, 2014). On the day of the calorimetry measurement, each steer was

placed in a stanchion where the steer's head was placed in a portable respiration headbox. The daily meal was provided, and the box was sealed. Ambient air flow through the box was allowed to stabilize before CH_4 was determined. The portable respiration headboxes were 0.76 × 0.76 × 1.78 m and were constructed with an aluminum frame and covered with 5-mm clear acrylic sheets. Each box was fitted with a vinyl hood in a 28 × 117 cm opening that attached around the animal's neck to provide a seal between the box and animal. Methane concentration was determined by pulling air through the box across a temperature-compensated, dry test meter to determine air flow exiting the box. Air temperature and humidity were determined with a Pace temperature/relative humidity sensor attached to a Pace data logger (Pace Scientific Inc., Mooresville, NC). Proportional samples of air entering the box and leaving the box were constantly collected into gas bags to provide a composite air sample for the collection period. Gas bags were constructed of a polyethylene-aluminum-Mylar laminate. Methane concentrations were analyzed with a dual-range infrared analyzer (AR-60A, Anarad, Inc., Santa Barbara, CA). Differences in in vivo CH_4 production between positive and negative RG steers were tested with a multivariate model where RG was a fixed effect and previous 24-h feed intake was a covariate. Steer was considered to be the experimental unit. Analyses were run using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Least squares means ± SE are reported in the text and were considered to differ if $P < 0.05$.

In Vitro CH_4 Measurements

Rumen and cecum contents were obtained from steers at slaughter. Contents were transferred to the laboratory in a warm vacuum flask. Rumen fluid was strained through 4 layers of cheesecloth. The rumen fluid was allowed to settle for approximately 45 min in a 39°C water bath in which feed particles floated to the top and solids and protozoa settled to the bottom of the flask. Bacteria were then collected from the middle of the flask and transferred to a secondary flask and bubbled with O_2-free CO_2 for 3 min. The resulting rumen fluid and cecum content (40 mL) were anaerobically transferred to 160-mL serum bottles with medium containing 146 mg/L of K_2HPO_4, 138 mg/L of KH_2PO_4, 240 mg/L of $(NH_4)_2SO_4$, 240 mg/L of NaC_l, 50 mg/L of $MgSO_4{\cdot}_7H2_O$, and 32 mg/L of $CaCl_2$ (2:1 ratio of media to rumen or cecum fluid). The bottles were then capped with butyl rubber stoppers and aluminum seals. The bottles were gassed with 45 mL of H_2 to displace any potential oxygen at the start of the incubation. The serum vials were then placed in an agitating water bath at 39°C. After 2 h of incubation, the serum vials were removed from the water bath, and a 2-mL gas sample was collected and injected into a 10-mL glass vial fitted with a butyl rubber stopper and aluminum sealed for later analysis. The gas samples were collected using a 25-mL airtight glass syringe fitted with a Luer lock needle and a 3-way stopcock. Headspace volume was determined with a glycerol-lubricated airtight syringe after each sampling time to account for changes in gas volume. The headspace was always under pressure. The gas sampling process was repeated at 4, 6, and 8 h after incubation. Methane concentration was analyzed using an 8610C gas chromatograph (SRI Instruments, Torrance, CA). The gas chromatograph was equipped with helium ionization and thermal conductivity tubes. The instrument was configured for multiple gas analyses using a 10-port gas sampling valve with 1-mL injection loop and a 91-cm-long column-packed molecular sieve 5A. Gases were separated using He gases during a 4-min run. Three gas standard mixes (Scotty Specialty Gases, Plumsteadville, PA) were used nondiluted and diluted in N_2 to produce a range of gas concentrations for a standard curve. Each steer had 4 replicate bottles for both the rumen and cecum. Rate of gas production was determined by regressing CH_4 yield on time within bottle. Replicates were averaged within animal and collection site. Differences in in vitro CH_4 production between positive and negative RG steers within collection site were tested. Analyses were run using the GLM procedure of SAS (SAS Inst. Inc.). Least squares means ± SE are reported in the text and were considered to differ if $P < 0.05$.

Level of Methanogen 16S rRNA

The detailed procedure of the DNA extraction method is similar to that described by Yu and Morrison (2004). Briefly, 0.3 g of sample was centrifuged for 5 min at 16,000 × *g* 4°C to pellet the DNA and solids and then resuspended in 0.2 mL TE (Tris-EDTA) buffer. Cell lysis was achieved by bead beating 0.15 g of the resuspended sample in ZR BashingBead Lysis Tubes (Zymo Research Corp., Santa Ana, CA) using the TissueLyser II (Qiagen, Hilden, Germany) for 3 min at 21 Hz in the presence of 4% (wt/vol) sodium dodecyl sulfate (**SDS**), 500 m*M* NaCl, and 50 m*M* EDTA. After mechanical and chemical lysis, ammonium acetate was used to precipitate and remove the impurities and the SDS, along with isopropanol precipitation for the recovery of the nucleic acids. Genomic DNA was then purified with RNase and proteinase K treatment, followed by the use of QIAamp columns from the Qiagen DNA Stool Mini Kit (Qiagen). Genomic DNA concentration was determined using a Nanodrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Table 1. The 16S rRNA primers used for real-time quantitative PCR analyses

Bacterial population	Name	Direction	Nucleotide sequence	Size, bp	Reference
Total bacteria	27f	Forward	AGAGTTTGATCCTGGCTCAG	484	Lane, 1991
	519r	Reverse	GTATTACCGCGGCTGCTG		Turner et al., 1999
Total methanogens	uniMet-1f	Forward	CCGGAGATGGAACCTGAGAC	165	Zhou et al., 2009
	uniMet-1r	Reverse	CGGTCTTGCCCAGCYCTTATTC		Zhou et al., 2009
Methanomicrobiales (Order)	MMB282f	Forward	ATCGRTACGGGTTGTGGG	506	Yu et al., 2005
	MMB832r	Reverse	CACCTAACGCRCATHGTTTAC		Yu et al., 2005
Methanobacteriales (Order)	MBT857f	Forward	CGWAGGGAAGCTGTTAAGT	344	Yu et al., 2005
	MBT1196r	Reverse	TACCGTCGTCCACTCCTT		Yu et al., 2005
Methanosarcina (Genus)	MB1b	Forward	CGGTTTGGTCAGTCCTCCGG	271	Shigematsu et al., 2003
	SAR835r	Reverse	AGACACGGTCGCGCCATGCCT		Shigematsu et al., 2003
Methanobacterium (Genus)	Mbt-202f	Forward	CGCCTAAGGATGGATC	197	Nelson, 2011
	Mbt-399r	Reverse	TAAGAGTGGCACTTGGGK		Nelson, 2011
Methanobrevibacter ruminantium + *Mbb. cuticularis*	Mbb-g1-f	Forward	GCTAATACYGGATAGATRAT	166	Skillman et al., 2006
	A329r	Reverse	TGTCTCAGGTTCCATCTCCG		Yu et al., 2008
Methanobrevibacter smithii + *Mbb. wolinii* + *Mbb. thaueri* + *Mbb. gottschalkii* + *Mbb. woesei*	Mbb-g2-f	Forward	GATAATACTGGATAGGCAAT	166	Skillman et al., 2006
	A329r	Reverse	TGTCTCAGGTTCCATCTCCG		Yu et al., 2008

A total of 5 ng of total DNA from rumen fluid, cecum content, or rectal content samples were used in 10-μL reactions containing 1XSYBR green master mix (Roche, Indianapolis, IN) and 1 μ*M* of each forward and reverse oligonucleotide primer (Table 1). Primer sets target conserved regions of the 16S rRNA gene. Each oligonucleotide set was used in a separate real-time PCR reaction. Real-time quantitative PCR (**qPCR**) was performed in triplicate using LightCycler 480 (Roche, Indianapolis, IN) at 95°C for 5 min followed by 50 cycles at 95°C for 10 s, 54°C for 10 s, and 72°C for 10 s and a final melting curve from 65°C to 97°C.

To quantify the bacterial and methanogen copy number from each sample, standard curves were generated for each primer set. Briefly, PCR products were generated from each primer set and were subsequently cloned into the Topo vector and transformed into One Shot TOP10 competent cells using the Topo TA cloning kit (Invitrogen, Carlsbad, CA) to use as reference plasmids for quantifying total bacteria, total methanogen, and specific methanogen subspecies. DNA from transformed cells was isolated using the QIAprep spin miniprep kit (Qiagen) and confirmed by agarose gel electrophoresis. DNA concentration was quantified with a Nanodrop 8000, and copy number was calculated with the Thermo Scientific DNA copy number calculator (http://www.thermoscientificbio.com/webtools/copynumber/). Plasmid control samples were diluted to concentrations of 10^2 through 10^8 copies of plasmid/μL and were subsequently stored at −20°C in aliquots. The calculation of copy number of total bacteria, total methanogens, or methanogen subspecies from each sample was performed using the appropriate standard curve calculated by LightCycler 480 software (release 1.5.0) with the Abs Quant/Fit Points analysis program.

The experimental design for the level of methanogens was a 2 × 3 factorial. The main effects were RG (positive and negative) and gastrointestinal location (rumen, cecum, and rectum). The statistical model included RG, gastrointestinal location, and the interaction as fixed effects. Steer was considered to be the experimental unit. Analyses were run using the GLM procedure of SAS (SAS Inst. Inc.). Least squares means ± SE are reported in the text and tables. Means were tested using least squares pairwise differences and were considered to differ if $P < 0.05$.

RESULTS

Residual gain was calculated from the regression of BW on DMI; $f(x) = (0.1262 \pm 0.0128)x + (25.7 \pm 9.9)$ ($R^2 = 0.43$; Fig. 1). The mean DMI for the entire feeding period was 772 ± 90 kg. Equipment failure resulted in one steer in each treatment group not having an enteric CH_4 measurement. Enteric methane production did not differ ($P = 0.11$) between the positive RG (112 ± 13 L/d) and the negative RG (74 ± 13 L/d) steers. In vitro rumen methane production did not differ between positive RG ($64.26 \times 10^{-5} \pm 10.85 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}) and negative RG ($61.49 \times 10^{-5} \pm 10.85 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}; $P = 0.86$). In vitro cecum methane production did not differ between positive RG ($4.24 \times 10^{-5} \pm 1.90 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}) and negative RG ($4.35 \times 10^{-5} \pm 1.90 \times 10^{-5}$ mmol·g^{-1} DM·min^{-1}; $P = 0.97$).

Methanogen 16S rRNA as a percentage of the total 16S rRNA bacteria did not differ between RG groups ($P = 0.18$). The methanogen 16S rRNA as a percentage of rumen fluid total bacteria 16S rRNA (5.3% ± 3.1%) did not

Table 2. Methanogen 16S rRNA groups expressed as a percentage of total methanogens 16S rRNA

Group	Rumen	Cecum	Rectum	SEM	*P*
Methanomicrobials	6.8	7.9	8.6	2.0	0.81
Methanobacteriales	76.2[a]	29.3[b]	9.1[b]	7.9	<0.001
Methanosarcina	8.4	2.9	10.7	3.1	0.19
Methanobacterium	0.7[a]	3.9[b]	2.7[b]	0.8	0.02
Methanobrevibacter ruminantium + *Mbb. cuticularis*	0.1	3.8	5.1	1.6	0.09
Methanobrevibacter smithii + *Mbb. wolinii* + *Mbb. thaueri* + *Mbb. gottschalkii* + *Mbb. woesei*	22.0[a]	36.5[a]	0.0[b]	5.1	<0.001

[a,b]Means differ within rows at $P < 0.05$.

differ from the methanogen 16S rRNA as a percentage of cecum content total bacteria 16S rRNA (11.8% ± 3.1%; $P = 0.14$). The methanogen 16S rRNA as a percentage of the rectum content total bacteria 16S rRNA (0.7% ± 3.1%) was not different from that of the rumen content ($P = 0.29$) but was less than that of the cecum content ($P = 0.01$). Methanomicrobiales 16S rRNA as a percentage of total methanogen 16S rRNA did not differ across sample sites ($P = 0.81$; Table 2); however, steers with positive RG (10.5% ± 1.6%) were more numerous than steers with negative RG (5.1% ± 1.6%; $P = 0.02$). As a percentage of the total methanogen 16S rRNA, 16S rRNA of Methanobacteriales ($P = 0.23$), *Methanobacterium* ($P = 0.60$), and the *Methanobrevibacter* group containing *smithii, wolinii, thaueri, gottschalkii,* and *woesei* ($P = 0.41$) did not differ between RG groups. Methanobacteriales and the *Methanobrevibacter* group containing *smithii, wolinii, thaueri, gottschalkii,* and *woesei* had a greater percentage of the total methanogen 16S rRNA in the rumen compared with the cecum and rectum, which did not differ from each other. *Methanobacterium* was a lesser percentage of the total methanogen gene 16S rRNA in the rumen compared with the cecum and rectum, which did not differ from each other. The group containing *Methanobrevibacter ruminantium* and *cuticularis* did not differ between RG groups ($P = 0.25$) and tended to differ with collection site. The percentage of 16S rRNA of *Methanosarcina* did not differ with RG group ($P = 0.42$) or collection site.

DISCUSSION

Methanogens are strictly anaerobic, making them difficult to culture (Smith and Hungate, 1958). The difficulty in culturing methanogens has made it hard to determine the relative proportion of methanogen species in the rumen. Using genomic sequence has allowed for inferring the relative abundance of methanogens in the rumen on the basis of 16S-rRNA copy number, but it does not ensure that they were viable. Mosoni et al. (2011), using genomic sequence, determined that methanogens were approximately 1% of the total bacteria in the rumen in sheep fed hay diets. In our study on a high-concentrate diet, total methanogens represented closer to 5% of the total bacteria measured by the 16S rRNA, which is similar to what Frey et al. (2010) found in a single dairy cow.

The cecum had a low potential to produce methane in vitro. Popova et al. (2013) reported similar findings in lambs fed wheat- and corn-based diets. In the Popova et al. (2013) study, they reported fewer methanogens in the cecum compared with the rumen. We did not find a difference in the relative percentage of total methanogen 16S rRNA compared with total bacterial 16S rRNA, which is inconsistent with the potential to produce CH_4. The presence of the 16S rRNA methanogen sequence does not ensure that there are equal viable cells in both the cecum and rumen. Frey et al. (2010), using qPCR with 16S rRNA, found genomic material attributed to methanogens in the duodenum of dairy cows. If a genomic sequence originating farther up the digestive tract is being detected in the cecum, then we may be overestimating methanogenic potential of the cecum on the basis of the presence of the 16S rRNA sequence. The proportion of 16S rRNA sequences that was methanogens in the rectum was 0.7%. Frey et al. (2010) found 1.3% of the 16S rRNA sequence in feces was attributed to methanogens.

Previous reports have identified *Methanobrevibacter* species as being the predominant methanogens in the rumen (Miller et al., 1986; Skillman et al., 2006; Zhou et al., 2010; Carberry et al., 2014). Our findings are consistent with the earlier observations in that we found a predominance of Methanobacteriales, which includes *Methanobrevibacter*. Methanogens classified in the group that contained *Mbb. smithii*, *Mbb. wolinii*, *Mbb*. *thaueri*, *Mbb*. *gottschalkii*, and *Mbb*. *woesei* were predominant in the rumen. *Methanobrevibacter* were predominant in the cecum as well but not in the rectum. The Methanobacteriales were the dominant group in the cecum, which is consistent with the findings of Popova et al. (2013) in lambs fed concentrate diets. Methanogen profiles in the rectal contents in general did not follow profiles in the rumen and cecum. These findings suggest that methanogen profiles in the feces are not predictive of what is present in the rest of the digestive tract.

We did not observe differences in total methanogens between steers classified as having positive or negative RG. Steers with a negative RG had a higher proportion of Methanomicrobiales. The observation of a lack of difference for methanogens between RG classifications is consistent with the absence of difference in enteric and in vitro CH_4 productions. Zhou et al. (2009, 2010) did not observe differences in total methanogens across cattle classified with different RFI but did observe a difference in the distribution of groups of methanogens across RFI classifications. They observed a shift in *Methanobrevibacter* and *Methanosphaera*. Animals classified to differ in RFI typically differ in feed intake. The reduction in enteric CH_4 production in animals with lower RFI can be associated with the reduction in feed intake (Hegarty et al., 2007). Although selecting for cattle that differ in RFI can change the relative proportions of methanogens, it is not clear that the methanogenic potential has changed. In our study, we selected animals that differed in RG at a common feed intake, and if methanogen population shifts are a function of feed intake, we would not expect to observe differences. The observed shifts in methanogen populations in cattle with different RFI may be a function of the differences in the level of feed intake affecting rumen passage rate and digestion.

Differences in individual CH_4 production may be associated more with the physical characteristics of the animal than with the profiles of methanogens in the rumen. Goopy et al. (2014) reported that sheep with smaller rumens and rumen retention time produce less CH_4. If selection for cattle that differ in RG or RFI results in increased efficiency as a result of increased metabolic efficiency, we would not expect a decrease in CH_4 production per unit of feed. However, if the increased efficiency is the result of an increase in digestion, then CH_4 production may increase with increased feed efficiency.

Our findings do not support the idea that a lower CH_4 production is the mechanism that contributes to variation in RG on high-grain diets. Methane is a product of fermentation during the digestive process. Previously, we did not find that differences in DM digestibility contribute to BW gain in cattle fed high-grain diets (Davis et al., 2014). The differences in RG at a common feed intake may be more a function of metabolic differences in efficiency than differences in digestive efficiency.

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